January 16, 04

Indirect Immunofluorescence for *in situ* localization of SmGPCR in *S. mansoni*

The antibody developed to localize SmGPCR was specifically raised against il3 (the 3rd intracellular loop) and thus, the samples require permeablization first to allow the antibody entry. Thus, **Triton X-100** was used for this purpose in all steps.

*** Fixative solution:** acetone (stored in the freezer) in water or acetone in PBS (3ml cold acetone +10ml water).

*** Blocking solution:** 5% Goat serum in PBS, pH 7.4 + 0.5% Triton X-100

*** Washing buffer:** PBS

*** 1ry Ab solution:** 1:100 of rabbit αSmGPCR IgG in 0.5% Triton X-100 in PBS.

*** 2ry Ab:** 1:300 of Goat α rabbit IgG –fluorescin labeled in 0.5% Triton X-100 in PBS.

Positive rabbit α SmGPCR IgG: The lyophilized rabbit α SmGPCR IgG is reconstituted in 400µl 1X PBS, pH 7.4 and used in 1:100 in 5%goat serum +0.5% TritonX-100/PBS.

Preadsorbed 1ry Ab: the positive antibody is incubated with 1mg/ml recombinant purified il3 antigen in 1:1 ratio for 3hours (or overnight) before using it in the Ab step (#5, below) at 1:50, instead of 1:100.

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(1) Cercarial bodies (after or before acetabular gland evacuation) or schistosomula (after washing out of the culture medium) are fixed in a very cold acetone in water (or PBS) in 15ml falcon tube for 2-3 days at 4°C in the end-over-end rotor.

(2) Wash with 1X PBS to get rid of acetone 5times (each is for 10minutes incubation and 10min centrifugation at 1400 rpm using Sorvall centrifuge).

(3) Block in 5% goat serum and 0.5% Triton X-100 in PBS for 2days at 4°C in the end-over-end rotor.

(4) Wash for 5times as in step 2.

(5) Incubate with the **1:100** positive 1°ry antibody (or ***preadsorbed** one*) in 5%goat serum and 0.5% Triton X-100/PBS or use only 5%goat serum and 0.5% Triton X-100/PBS as a negative control (i.e. absence of 1ry Ab.) for **3-4 days**, at 4°C in the end-over-end rotor.

(6) Repeat step 2.

(7) Add the commercial labeled 2°ry Ab (goat α rabbit IgG –fluorescin labeled) as **1:300** in 5% goat serum and 0.5% Triton X-100/PBS for **2-3days**, at 4°C in the end-over-end rotor. Wrap with aluminum foil (since the labeled 2ry Ab is light sensitive).

(8) Repeat step 2. Beware to cover the falcon tubes with aluminum foil during washings to protect the labeled samples from bleaching.

(9) Last step of washing, centrifuge in eppendorf tubes using our lab bench centrifuge to collect the pellet for easily mounting.

(10) Mount a drop of the pellet in a clean slide, add a drop of mounting medium (sigma) and add the cover slip and seal it with nail polish to protect from rapid bleaching. Keep away from light.
